U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-294 303

Report of the Workshop on Biological Screening Tests Held at Las Vegas, Nevada on September 12-14, 1977

Tracor Jitco, Inc, Rockville, MD

Prepared for

Environmental Monitoring and Support Lab, Las Vegas, NV

Jan 79

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/9-79-004		PB294303

4. TITLE AND SUBTITLE	5. REPORT DATE
REPORT OF THE WORKSHOP ON BIOLOGICAL SCREENING TESTS	January 1979
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Charles A. Bicking	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Tracor Jitco, Inc. Rockville, Maryland 20852	1AA601
	11. CONTRACT/GRANT NO.
	CB-7-0913-B
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency-Las Vegas, NV Office of Research and Development Environmental Monitoring and Support Laboratory Las Vegas, NV 89114	Final
	14. SPONSORING AGENCY CODE
	EPA/600/07

15. SUPPLEMENTARY NOTES
EMSL-LV Project Officer for this report is John A. Santolucito.
Commercial Telephone (702) 736-2969, x 276 or FTS 595-2969, x 276

16. ABSTRACT

This report contains recommendations for selecting substantially predictive biological screening tests. The large number of chemicals which can potentially impact human health and the environment precludes the complete testing of each substance. In order to effect preliminary chemical hazard ranking, initial tests must be standardized and validated and the necessary quality control practices and techniques developed and implemented.

This report contains recommendations for selecting substantially predictive biological screening tests upon which the Agency's quality assurance resources may be initially concentrated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicology Hazardous materials Physiological effects Ecological effects Environmental biology	Biological screening tests	06T 06F

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	52
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	PC A04/MF A01

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1979-749-903/12

United States
Environmental Protection
Agency

Environmental Monitoring
and Support Laboratory
P.O. Box 15027
Las Vegas NV 89114

EPA-600/9-79-004
January 1979

Research and Development

PB 294303

EPA

Report of the Workshop on Biological Screening Tests

Las Vegas, Nevada
September 12-14, 1977

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

inspections, and monitoring (under this law as well as other laws and regulations).

It is anticipated, then, with the qualifications above, that the following types of chemical and physical data should be supplied by the manufacturer:

I. Product

A. Chemical: The following data should be presented for each chemical component accounting for 1 percent or more of the total.

1. Identity and molecular structure

2. Percent of each chemical compound in the product and the anticipated range and variability for each

3. Analytical methods used in the identification of each chemical compound in the product and in its quantification

B. General characterization (e.g., physical state, color, odor, crystalline form if applicable)

C. Reference sample: A representative sample of the product should be submitted to the EPA. This sample could be used by the EPA for cross-checking data submitted by the manufacturer, for further analytical and biological tests, and for future reference (e.g., in evaluation of batch-to-batch variation in toxic impurities). It is recommended that the EPA establish a product repository for storing and distributing these reference samples.

D. Physical properties

1. Melting point (if solid)

2. Boiling point (if liquid)

3. Vapor pressure

a. At ambient temperature and pressure

b. Under anticipated conditions of use

4. Flashpoint

5. Polarity

6. Solubility

EPA-600/9-79-004
January 1979

REPORT OF THE WORKSHOP ON

BIOLOGICAL SCREENING TESTS

Las Vegas, Nevada
September 12 - 14, 1977

by
Charles A. Bicking, Editor
Tracor Jitco, Inc.
Rockville, Maryland 20852

Contract No. CB-7-0913-B

Project Officer
Dr. John A. Santolucito
Monitoring Systems Research and Development Division
Environmental Monitoring and Support Laboratory
Las Vegas, Nevada 89114

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
LAS VEGAS, NEVADA 89114

DISCLAIMER

This report has been reviewed by the Environmental Monitoring and Support Laboratory-Las Vegas, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound monitoring data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report contains recommendations for selecting substantially predictive biological screening tests. The large number of chemicals which can potentially impact human health and the environment precludes the complete testing of each substance. In order to effect preliminary chemical hazard ranking, initial tests must be standardized and validated and the necessary quality control practices and techniques developed and implemented.

This report contains recommendations for selecting substantially predictive biological screening tests upon which the Agency's quality assurance resources may be initially concentrated.

George B. Morgan
Director
Environmental Monitoring and Support Laboratory
Las Vegas

CONTENTS

Foreword iii

Abbreviations vi

Introduction 1

Conclusions and Recommendations 4

Report on Chemical/Physical Aspects of Toxicity Testing 8

Report on Whole Animal Acute and Sub-chronic Testing 15

Report on In-Vitro Testing 25

Report on Model Ecosystems 29

General Bibliography 36

List of Workshop Participants 42

Preceding page blank

LIST OF ABBREVIATIONS

ABBREVIATIONS

AOAC	-- Association of Official Analytical Chemists
ASTM	-- American Society for Testing and Materials
BOD	-- biological oxygen demand
CFR	-- Code of Federal Regulations
CNS	-- central nervous system
COD	-- chemical oxygen demand
CPSC	-- Consumer Products Safety Commission
CSL	-- Chemical Systems Laboratory
DHEW	-- Department of Health, Education, and Welfare
EMSL-LV	-- Environmental Monitoring and Support Laboratory-Las Vegas
EPA	-- U.S. Environmental Protection Agency
ERL	-- Environmental Research Laboratory
F2	-- second generation effects
FDA	-- Federal Drug Administration
FR	-- Federal Register
NIH	-- National Institutes of Health
OMTS	-- Office of Monitoring and Support
ORNL	-- Oak Ridge National Laboratory
OSHA	-- Occupational Safety and Health Administration
OTS	-- Office of Toxic Substances
P	-- primary production
PR	-- primary production/respiration ratio
R	-- respiration
TOC	-- total oxygen concentration
TOSCA	-- Toxic Substances Control Act
w/w	-- ratio of gaseous to particulate

INTRODUCTION

The Workshop on Biological Screening Tests was cosponsored by the Environmental Monitoring and Support Laboratory-Las Vegas (EMSL-LV) and the Office of Toxic Substances, U.S. Environmental Protection Agency (EPA).

The Workshop objective was to identify and recommend screening tests which are immediately applicable and substantially predictive of the impact of chemical pollutants on biological systems.

Attendees included representatives of EPA laboratories involved in biological research, individuals from a number of other governmental agencies engaged in similar work, academic experts in various fields of toxicity testing, and individuals from Tracor Jitco, Inc., of Rockville, Maryland, who had responsibility for convening the Workshop, assembling working group reports, and preparing this report. A list of the participants is included at the end of this document.

The Manufacturing Chemists Association was invited to send a representative to the Workshop and to give a presentation. Unable to do so, it recommended a paper by Astill et al., as representing suitable industry input on the subject matter of the Workshop: Astill, B. D., et al., 1977. A Tier Testing Scheme. Presented at the Toxicological Forum, Institute of Pathology, Aspen, Colorado, July 18-22, 1977.

As shown in the Workshop agenda on page 3, invited papers covering various areas of toxicity testing of interest to the Workshop were presented on the first day. The second and third days were devoted to separate meetings of working groups on Chemical/Physical Testing, Whole Animal Acute and Subchronic Testing, In Vitro Testing, and Model Ecosystems.

Although each of the working groups concluded that it was possible, within the brief duration of the meeting, to identify applicable tests in its area, the recommendation of a specific battery of tests was impractical for a number of reasons. Among the reasons were: (1) the small amount of information available that correlates short-term tests with long-term effects of interest to the EPA; (2) the developmental stage in which a number of promising tests remain; and (3) the desire of working group participants to obtain a wider consensus within the scientific community on which of the available tests should be selected.

The working groups felt that the range of substances of interest to the EPA is so wide that no single set of tests could be prescribed which would meet all conditions. They also felt there was a need to consider not only toxicity to animals and humans (health effects) but also adverse effects in

the whole environment (ecological effects).

Most of the Workshop participants felt that their view of the EPA's need was too limited to warrant their recommending specific tests, and that the EPA had the responsibility for selecting a battery or batteries of tests from the lists the Workshop participants supplied. This was not meant to preclude further assistance from the scientific community in filling information gaps.

This report presents the conclusions and recommendations drawn from individual reports of each working group which are also included.

AGENDA FOR THE WORKSHOP ON BIOLOGICAL SCREENING TESTS

General Session

Monday, September 12, 1977

Welcome to Laboratory - George B. Morgan, Director, EMSL-LV

Opening Remarks - Albert C. Trakowski, Jr., OMTS, and William M. Upholt, OTS

Workshop Charge - John A. Santolucito, EMSL-LV

Testing of Generic Groups of Chemicals - John M. Bryant, OSHA

Biological Monitoring of Available Toxic Materials in Soil - Robert D. Rogers, EMSL-LV

Applicability of Animal-to-Human Correlation from Tissue Culture - John F. Lontz, VA

Approaches to Screening Agents for Mutagenicity - Sidney Green, Howard University

Whole Animal Toxicity Testing - Bernard P. McNamara, CSL

Model Ecosystems - Sidney Draggan, ORNL

Notices and Instructions for Working Groups - John Santolucito, EMSL-LV

Working Group Sessions

Tuesday, September 13, and Wednesday, September 14, 1977

I. Chemical/Physical Aspects of Toxicity Testing

II. Whole Animal Acute and Subchronic Testing

III. In Vitro Testing

IV. Model Ecosystems

CONCLUSIONS AND RECOMMENDATIONS

CHEMICAL/PHYSICAL TESTING

The report of the Working Group on Chemical/Physical Aspects of Toxicity Testing outlines the data that a manufacturer may be expected to submit in complying with the Toxic Substances Control Act (TOSCA). These data will be useful in evaluating the potential environmental impact of the chemical, in establishing the priorities for biological testing of chemicals, and in selecting appropriate biological test methods for each chemical.

Not every test listed in the report will be applicable to every product examined as a result of a requirement of the TOSCA. The parameters measured might vary greatly depending upon the nature of the substance and the problems of evaluating its ultimate environmental impact. Most of the data required should be available from standardized analytical tests and from good manufacturing practices.

Structure/activity correlations have been attempted for many chemical classes and, where data are available, they should be used judiciously and to the extent possible in selecting biological tests to be performed.

In compliance with Sections 4, 5, and 8 of the TOSCA, representative samples of proposed new products should be submitted to the EPA and it is recommended that, should such sample collection be activated, the EPA establish a product repository for storing and distributing these reference samples.

There is a need for standardized tests for measuring biodegradability.

WHOLE ANIMAL TESTING

National laws and regulations indicate that it may be necessary to test for any or all short- or long-term toxicological effects on body organs or functions. Test areas and sources of test methods are tabulated in the report of the Working Group on Whole Animal Acute and Subchronic Testing.

The practical situation of use may make the degree of exposure and risk very different for different chemicals. Not all compounds need to be tested for every effect and it is possible that some compounds may require no testing.

The Working Group did not attempt to assess the degree of risk; instead it outlined, within current scientific knowledge, those tests which were necessary and acceptable, leaving it to the EPA to specify the tests required

for a chemical, based on use information supplied by manufacturers.

Problems associated with adherence to a strict experimental protocol for each class of chemicals include:

- Pre- and post-natal exposure
- Selection of species and strain
- Sex, age, pre-existing or intercurrent disease
- Route of administration
- Frequency and level of dosing
- Dose-response relationships
- Interaction with other chemicals and environmental factors which may produce synergistic, potentiating or antagonistic effects.

Aquatic Environment Testing

An outline is presented showing, for various aquatic species, acute and chronic tests with comments regarding their availability and use. The tests are classified as readily available, or as new but promising. No specific recommendations are made on tests to be run, but the information in the outline is adequate to enable the EPA to select a screening battery of available tests which can be used to assess the toxicity of a chemical to a variety of aquatic species.

Behavioral Toxicology

Relatively little data have accumulated on the neurophysiologic and behavioral effects of environmental chemicals. However, standardized tests are routinely used in screening drugs for central nervous system (CNS) effects and could be similarly applied to environmental pollutants. A screening might include, therefore, activity changes, objective signs, reflex changes, elicited responses, and body weight changes.

These screening tests should be followed by study of delayed effects using longitudinal research design which is well standardized and reported.

Tests requiring minimal instrumentation and training time include motor activity using a photocell cage or an activity wheel and Sidman avoidance performance. Sensory function tests are also available.

Attention should be given to the feasibility of using behavioral tests in a first-screening battery of tests for environmental toxicants. Rapidity of response, relatively low cost, ease of administration, and repeatability, all support this approach.

IN VITRO TESTING

The Working Group on In Vitro Testing focused its attention on endpoints of mutagenicity, carcinogenicity and cytotoxicity. It was agreed that short-term testing cannot be substituted for whole animal testing in any of these

three areas at present.

In mutagenicity, full source documents exist evaluating the correlation of short-term results with known in vivo results. In addition to published articles, more recent drafts have summarized the state-of-the-art in mutagenicity testing methodologies. No such full source documentation is available for carcinogenicity. Procedures available for consideration consist, therefore, of short-term tests already in use in the mutagenicity area, plus neoplastic cell transformation assays.

In cytotoxicity, evaluation of acute cellular toxicity in vitro provides information regarding potential in vivo activity of chemicals, prosthetic materials and devices, and biomedical implants. Although it would be very difficult to replicate the entire range of cellular responses of the intact animal by use of tissue culture methods, certain target cell types lend themselves readily to maintenance in vitro. Exposure of these cell types to chemical substances provides information on cellular toxicity and metabolism.

It was the consensus of the Working Group that in vitro assay or short-term testing cannot be predictive for risk assessment of human populations. In addition, this group could also agree with the following statements:

- The source documents and drafts on mutagenicity testing contain the current state-of-the-art for a number of test systems. This area is well explored in methodology and is documented.
- Toxicological decisions (e.g., carcinogenicity) are usually on non-human studies; the same criteria should be applied to decisions in the area of mutagenesis.
- The available procedures for detecting and characterizing the effects of chemical mutagens are based upon our understanding of mutational processes. Therefore, decisions in chemical mutagenesis should now be considered as an integral area of toxicology. Further, as in all other areas of toxicology, there is a need for continual monitoring and upgrading of existing procedures. When interpreting the utility of short-term tests, we must know the distribution of a chemical, specifically to the gonads, in concert with applicable somatic effect, in vivo or in vitro, including gene and chromosome effects; only then can we rely on such testing as indicators of potential genetic hazard.

In summary:

- Available short-term mutagenicity tests for use as an aid in indicating potential carcinogenic hazard cannot at present stand alone to identify a carcinogen--long-term animal assays are still necessary.
- Neoplastic cell transformation assays, as yet not included in mutagenicity studies, are a grouping of in vitro tests that are still under evaluation.
- The whole area of cocarcinogenesis and promotion needs to be recognized.

- There is a possibility that different batteries of tests should be set up for different classes of chemicals. For example, the Office of Pesticide Programs (OPP) battery for pesticides is considered to be very good.

The Working Group made the following recommendations: (1) convene a group of experts to specifically consider the applicability of the Pesticide Guidelines to the needs of the Office of Toxic Substances in using short-term tests as indicators of mutagenic potential of chemicals, and (2) interact with the other regulatory agencies [Federal Drug Administration (FDA), Occupational Safety and Health Administration (OSHA), and Consumer Products Safety Commission (CPSC)] in the development of a technology assessment document [along the lines of the EPA Office of Pesticide Guidelines and the CPSC and the Department of Health, Education, and Welfare (DHEW) mutagenicity documents] to evaluate the status and use of short-term tests as aids in evaluating the carcinogenic potential of chemicals.

MODEL ECOSYSTEMS

According to the report of the Working Group on Model Ecosystems, screening tests in ecosystems were understood to be concerned with small replicas (microcosms) of natural systems constructed either artificially or taken intact from the field. These microcosms alone should not be expected to provide assessments of chemical hazards to the environment.

Initial assessment might best be accomplished using chemical benchmark information, simple short-term laboratory tests (e.g., EC_{50}, LD_{50}, or behavioral test) and mathematical models of chemical behavior. Microcosm testing provides information on chronic, long-term effects of hazardous chemicals on fundamental natural processes such as energy flux, nutrient cycling and homeostatic properties, and on species interactions.

The report includes tables giving information about microcosms that have been constructed and how they have been used. Microcosms generally are tailored to specific ecosystems, and questions about processes and observed chemical behavior usually are not amenable to generalization to ecosystems. Thus, there is no such thing as a standard microcosm.

A microcosm test should not be expected to have lower parameter variability than that of a natural environment. This variability is essential to adequate simulation of the natural environment and may provide data within the range of actual environmental occurrences. Standards for variability of replication do not exist. It is not well known to what extent construction of microcosms distorts field conditions, and methods for extrapolation from the microcosm to the field are still being debated.

Microcosm tests should address specific questions and data needs. Therefore, it is suggested that, based on the ecosystem type, tests of proven success be selected to meet the need.

REPORT ON
CHEMICAL/PHYSICAL ASPECTS OF TOXICITY TESTING

INTRODUCTION

Under Sections 5 and 8 of the Toxic Substances Control Act, a manufacturer may be required to submit chemical and physical data on the proposed new product and/or process to assist the Administrator in evaluating the possible risk to man and the environment associated with the manufacture, processing, distribution, use, and disposal of the product. This Workshop has attempted to catalog some of the important types of chemical and physical data that may be needed for risk assessment.

It is expected that these data will be submitted by the manufacturer with the initial notification. The data will be used in the evaluation of the anticipated magnitude of human exposure to the product and the byproducts of its manufacture, use, and dispersal into the environment and the potential effects of the product and its byproducts on human health and the environment. It is expected that some of these data may be of use in the selection of relevant biological tests that should be performed on the product and/or its byproducts. Structure/activity correlations have been attempted for many chemical classes and should be used judiciously and to the extent possible in the selection of biological tests that will be performed.

INFORMATION TO BE SUPPLIED BY THE MANUFACTURER

It is recognized that some of these data may not be pertinent or readily accessible for some new products or processes. An effort has been made, however, to limit the data requested to those which should be available from standardized quantitative tests and from good manufacturing practices. Thus, it is anticipated that the Administrator will require these data, where applicable, for a full evaluation of potential risk.

Much of this information would be required eventually, in any case, under other laws and regulations administered by the EPA. The intent here is to ensure that this information is gathered, reviewed, and evaluated to the extent possible, before the production process begins, so that situations presenting unacceptable risks can be prevented, rather than corrected after damage has resulted.

It also is recognized that, in some cases, these data will be based on extrapolation from pilot plant or even smaller scale operations, with considerable attendant uncertainty. The Act recognizes this problem and provides for follow-up after the process goes on line in the form of reports,

a. In water as a function of pH

b. In hexane

7. Octanol-water partition coefficient (polar/non-polar)

8. Conductivity of aqueous solutions

E. Chemical properties

1. Oxidation and reduction potentials (and product if known)

2. Rate of hydrolysis (and products if known)

3. Photochemical reactions (oxidation, etc.)

4. Electrophilicity

5. Differential thermal analysis, with identification of major off-gases

6. Chelating factor

7. Chemical oxygen demand (COD) (of aqueous solutions)

8. Chlorine demand (of aqueous solutions)

9. Corrosion

F. Biochemical properties

1. Biochemical tests: A suitable battery of in vitro biochemical tests should be devised that would assist in postulating the major metabolic processes and products in animals and man.

2. Biodegradability: Information on biodegradability is of obvious importance and should be provided whenever possible. The Working Group noted the need for standardized tests for this parameter.

II. Process: A complete quantitative mass balance for the manufacturing process should be provided, including identification and quantification of starting materials, products, byproducts, and anticipated emissions and effluents. Process chemistry should be outlined schematically, along with a careful description of pollution control technology and anticipated performance standards. See Paragraph III for emission and effluent information requirements.

III. Emissions and Effluents

A. Emissions (air)

1. Total (gross) emission

a. Ratio of gaseous to particulate (w/w)

b. Particle-size distribution

c. Solubility in water

2. Individual components: The following data should be presented for each chemical component in the air emissions accounting for 1 percent or more of the total (excluding air), by weight:

a. Chemical structure

b. Percent of total emission

c. Amount in pounds (kilograms) per day at capacity

d. Chemical properties

(1) Oxidation and reduction potentials (and products if known)

(2) Photochemical reactions (oxidation, etc.)

(3) Electrophilicity

e. Biochemical properties (see Paragraph I.F.1.)

B. Effluents (Water)

1. Total (gross) effluent

a. pH

b. Turbidity

c. Conductivity

d. Chemical oxygen demand (COD)

e. Biological oxygen demand (BOD)

f. Total oxygen concentration (TOC)

g. Chlorine demand

2. Individual components: The following data should be presented for each chemical component in the water effluent accounting for 1 percent or more of the total (excluding water), by weight:

   a. Oxidation and reduction potentials (and products if known)

   b. Solubility

      (1) In water as a function of pH

      (2) In hexane

   c. Partition coefficient (polar/non-polar)

   d. Electrophilicity

   e. Biodegradability

   f. Biochemical properties (see Paragraph I.F.1.)

IV. Environmental Degradation Products: To the extent possible, the following information should be submitted on all known and suspected products of environmental degradation of the product itself and important byproducts (see Paragraph III.A.2 and III.B.2).

   A. Chemical structure

   B. Source (degradation process) and where found (air, water, soil; near manufacturing, formulating plants; in homes, etc.)

   C. Anticipated quantities

   D. Rates of appearance and of further degradation

   E. Biodegradability

   F. Leachability (from typical soils)

   G. Solubility

      1. In water as a function of pH

      2. In hexane

   H. Partition coefficient (polar/non-polar)

   I. Photochemical reactions

V. Waste Disposal: Recognizing the ultimate necessity of disposal of most chemical products at some point in time and the attendant potential risks to human health and the environment, the following information on anticipated and recommended disposal practices should be submitted, where applicable.

A. Solid waste

1. Anticipated nature and composition of solid wastes

2. Combustion

a. Conditions required for complete combustion

b. Products of complete and of incomplete combustion

3. Sanitary Landfill

a. Likely leachates; relative quantities

b. Likely volatiles

4. Recommended disposal procedures for solid wastes

B. Liquid waste

1. Anticipated nature and composition of liquid wastes

2. Combustion (see Paragraph A. 2 above)

3. Sanitary landfill (see Paragraph A. 3 above)

4. Discharge into sewage system

a. Toxicity/compatibility with sewage treatment systems; at what levels?

b. See Paragraph III.B.

5. Discharge into receiving waters (navigable waters)

a. See recommendations of Aquatic Toxicology Testing Group.

6. Recommended disposal procedures for liquid wastes

EPILOG

This discussion of chemical and physical data which may be required for risk assessment has focused on the product and the manufacturing process. However, it is clear that the subsequent formulation, processing, distribution, and use of the product will often constitute the major portion of the

risk to human health and the environment presented by a new product or process. The Toxic Substances Control Act requires the manufacturer to provide such information as is available on anticipated uses with the initial notification, but much of this will be conjecture until the product is actually marketed.

Therefore, additional data will undoubtedly be required on formulation, processing distribution, and uses after marketing. This might include a periodic market profile and an evaluation of the potential for human exposure from release of the chemical substance to the environment. Each major use and each stage of the formulation and distribution process should be evaluated.

It is anticipated that not every test would be applicable to every product examined as a result of requirements of the Toxic Substances Control Act. Indeed, the parameters might vary greatly, depending upon the nature of the substance and the techniques available for evaluating its ultimate environmental impact. Standard, or at least widely accepted, methods for measuring many of the listed properties currently exist in the literature. Standard reference sources such as the Association of Official Analytical Chemists (AOAC) and the American Society for Testing and Materials (ASTM) list recommended techniques for determinations such as conductivity, polarity, BOD, COD, etc.

If standard or reference methods are not available, suitable techniques may be found in the technical and experimental literature. In many cases, new methods will have to be devised to make the required measurements.

REPORT ON
WHOLE ANIMAL ACUTE AND SUBCHRONIC TESTING

INTRODUCTION

In order to protect the public against hazards resulting from exposure to environmental chemicals, it is necessary to know the short- and long-term (acute, subchronic, and chronic) toxicological effects of the chemical upon all organs and functions of the exposed subjects. The practical situation of use may make the degree of exposure and risk very different for different chemicals. Not all compounds need to be tested for every effect and it is possible that some compounds may require no testing.

The Toxic Substances Control Act provides for submission by the manufacturer to the EPA of information describing the merits and value of the chemical, the nature of usage, the amount to be produced, the size of the population at risk, expected frequency and duration of exposure, any toxicity data, predictions of hazard, and protocols.

On the basis of the information submitted, the EPA should specify the tests required for a chemical within a given use or situation. The EPA can specify the test methodology on the basis of the "state-of-the-art" and established test methods. Within an established method, the EPA should reserve the authority to specify the nature of the test sample, animal species, routes of administration, dose levels, solvents, diluents, dilution levels of test solutions, frequency and duration of dosing, and quality assurance procedures. These modifications ensure that the methodology is appropriate to the operational situation.

The purpose of the Working Group is to outline, within current scientific knowledge, those tests which are necessary and acceptable to demonstrate the presence of toxic effects in body organs or functions. The adequacy of existing data should be determined by the EPA. The tests used to supply the needed information should be selected from sources given below.

GUIDELINES FOR TOXICOLOGICAL TESTING

Requirements and guidelines for toxicological testing have been advanced by regulatory agencies and expert groups having official or quasi-official sponsorship or acceptance. They include the following:

1. Code of Federal Regulations (CFR) 46, Shipping. Jan. 1, 1972, para. 146.25-10.
2. CFR 49, Transportation. Parts 100-199, Jan. 1972, para. 173.343.

3. CFR 40, Protection of the Environment. Jan. 1, 1972, para. 162.8.
4. Federal Register (FR), Vol. 38, No. 187. Part II. Consumer Products Safety Commission, Federal Hazardous Substance Act Regulation, Revision and Transfer. Sept. 27, 1973.
5. FR, Vol. 40, No. 129. Part II, para. 162.8. Environmental Protection Agency Pesticide Program. Registration, Reregistration and Classification Procedures. July 3, 1975.
6. Appraisal of the Safety of Chemicals in Food, Drugs, and Cosmetics. 1959. Association of Food and Drug Officials of the U.S. Editorial Office, 2411 N. Charles Street, Baltimore, MD.
7. National Academy of Sciences-National Research Council. 1964. Principles and Procedures for Evaluating the Toxicity of Household Substances. Publication 1138.
8. National Academy of Sciences-National Research Council. Revised, 1977. Principles and Procedures for Evaluating the Toxicity of Household Substances. Publication 1138.
9. Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: Panel on Reproduction Studies in the Safety Evaluation of Food Additives and Pesticide Residues. Toxicol. and Appl. Pharmacol. 16: 264-296. 1970.
10. National Academy of Sciences-National Research Council. 1975. Principles for Evaluating Chemicals in the Environment.
11. Department of Health, Education and Welfare Committee to Coordinate Toxicology and Related Programs. April, 1977. Approaches in Determining the Mutagenic Properties of Chemicals: Risks to Future Generations.
12. Department of Health, Education and Welfare. Guidelines for Carcinogen Bioassay in Small Rodents. (DHEW) Publication No. (NIH) 76-801.
13. R. M. Graziano's Tariff No. 25. Hazardous Materials Regulations of the Department of Transportation including specifications for Shipping Containers. para. 173.343, 173-240. July 28, 1972.
14. CFR 21. Food and Drugs. Parts 147 to 199, para. 191.1. Jan. 1, 1972.
15. Health, Education, and Welfare - Canada. 1975. The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity. p. 28.
16. FR Vol. 40, No. 123. Environmental Protection Agency Pesticide Program, Guidelines for Registering Pesticides in the U.S. Part II, para. 162.81. Proposed Rules. 1975.
17. National Institutes of Health, Third Printing, 1977. Guide for the Care and Use of Laboratory Animals. DHEW NIH 74-23.

TOXICOLOGICAL TEST AREAS

Established laws (National Environmental Policy Act of 1969, Clean Air Act Amendments of 1977, Toxic Substances Control Act 1976) and the guidelines for toxicological testing which have been established or proposed by the Food and Drug Administration, Department of Transportation, Environmental Protection Agency, Consumer Products Safety Commission, National Cancer Institute and the National Academy of Sciences-National Research Council indicate that it may be necessary to test for any or all short- or long-term toxicological

effects on body organs for functions. Test areas appropriate to the guidelines are given in Table 1.

The table also cites the references, on pages 15 and 16, to acceptable methodologies which may be used to evaluate toxicological effects on the designated organ, organ system, or physiological function.

TABLE 1. TOXICOLOGICAL TEST AREAS

Toxicological Area	Reference
I. Acute Toxicity (single dosing)	1,2,3,4,5,6,7,8,10,14,15
A. General Toxicology	
1. LD_{50} (by appropriate routes)	1,2,3,4,5,6,7,8,10,14,16
LC_{50}	7,8,16
2. Clinical signs	5,6,8,10,16
3. Autopsy findings (gross)	5,6,8,10,16
B. Local Tissue Effect	
1. Skin and eye irritation	1,2,3,4,5,6,7,8,10,12,14,16
2. Skin sensitization test	4,5,6,8,10,14,16
II. Subchronic and Chronic Toxicity Tests	
A. Metabolism	6,8,10,16
B. Pharmacokinetics	6,10
C. Hematology	5,6,8,10
D. Clinical Chemistry Tests	5,6,8,10
E. Pathology	5,6,8,10,16
F. Pharmacodynamics	6
G. Behavior	6,8,10,16
H. Neurological Effects	6,10,16
I. Endocrinology	6
J. Reproduction	5,6,8,9,10,16
K. Teratogenesis	5,6,8,10,15,16
L. Mutagenesis	6,8,10,11,16
1. Dominant lethal	6,8,10,11,16
2. Cytogenetic	8,11,16
M. Carcinogenicity	5,8,10,12,15,16
1. Short-term	8,10,15
2. Long-term	6,10,12,15,16
III. Care and Use of Laboratory Animals	17

GENERAL PROBLEMS IN FOLLOWING THE TESTING PROCEDURES OUTLINE

The Working Group recognizes that certain problems would emerge as a result of strict experimental protocols for every class of chemicals that may warrant testing. Examples of these include: selection of species and strain of test organism, other variables such as sex, age, pre-existing or intercurrent disease, route of administration, frequency and level of dosing, and dose response relationships.

These and related problems are compounded in carcinogenicity testing because of the long latent period for development of cancer. They include pre- and post-natal exposure, differences in biochemical and biological competence between strains, sexes and species, interaction with other chemicals, and environmental factors which may produce synergistic, potentiating or antagonistic effects.

While some credence can be given to suspecting toxicity, it should not be assumed that qualitative and quantitative prediction of toxicity can be made on the basis of structural similarity to another compound; note for example, the difference in toxicity between kepone and mirex, isomers of benzene hexachloride, neurotoxic effects of organophosphates, and pharmacokinetic behavior of different halogenated compounds.

Finally, the Working Group recognizes the need for the inclusion of behavioral studies to reveal subtle dysfunctional effects on the central nervous system and the development of better animal models for extrapolation to humans.

MARINE ENVIRONMENT TESTING

I. Phytoplankton Bioassays

A. Laboratory Assessment: Short-term, many species. Growth Rate, Carbon-14 uptake, and Chlorophyll *a* are parameters. Methods already being routinely used for screening by EPA contractors.

B. Field Assessment: In situ Carbon-14, Adenine triphosphate, and Chlorophyll *a* measures. Standard oceanographic techniques. Routinely used in assessment of damage from powerplants.

These methods are applicable to acute hazard detection but offer, as presently applied, little information on bioconcentration and long-term hazard.

References: 1. Marine Bioassay Workshop Proceedings. 1974. API, EPA, MTS: Geraldine Cox, ed.; Marine Technology Society

2. Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-76-010; May 1976.

II. Zooplankton Bioassays

Laboratory Assessment: Acartia tonsa/Acartia clausi, sensitive indigenous marine species, presently used in Ocean Disposal and Dredge Spoil Programs.

Short-term static test is routinely run. Parameter is survival. Method amenable to other zooplankton (colcenoids).

Flow-through short-term assay method available but not tested outside the EPA's Environmental Research Laboratory-Narragansett (ERL-Narragansett). Potentially offers broad applicability and sensitivity.

III. Crustacean Larval Bioassay

Generally using larvae of macrocrustaceans. There are no routinely standardized methods here to point to. However, both static and flow-through exposure systems and protocols for Lobster larvae that are amenable to other decapod larvae are available.

IV. Static and Flow-through Bioassay Procedures for Grass Shrimp and Larvae (Paleomonetes)

Both procedures have been used effectively by in-house research laboratories but not extensively by contracting laboratories. Until further testing, they are not recommended as routine tests.

V. Static and Flow-through Bioassay on Mysids

This method, while recent in development, is being rapidly adopted for routine screening and also for whole life cycle chronic testing. It is currently being contracted and the EPA has interlab calibration in progress. Offers the opportunity to compare acute with "safe" levels of pollutant with short-life cycle species (18-30 days). Detailed methods are currently being prepared for publication by the EPA/Gulf-Breeze. Multiple parameter and bioaccumulation in the second generation effects (F2).

VI. Macroalgal Bioassays

New technique developed and applied at the ERL-Narragansett. Includes fertilization and growth. Developmental abnormalities are sensitive measures of acute hazard, while growth rate changes reflect chronic long-term exposure. Method also has field assessment potential. Method available from the ERL-Narragansett. Not currently contractable.

VII. Chronic Bioassay-Cyprinondon variegatus (Sheepshead Minnow)

Whole life cycle bioassay--widely used within the EPA and by contractors. Currently the only chronic exposure test system available using marine fish. Excellent for estimating a variety of parameters including bioconcentration and F2 effects.

VIII. Embryo-Fry Bioassay

Available for many species of fish, particularly suitable for indigenous species. Not widely used yet.

A. Cyprinodon variegatus (Sheepshead Minnow): Available from Gulf-Breeze, Florida, and contractable.

B. Flounder Species: Available from the ERL-Narragansett in winter and summer. Require special holding and spawning conditions. Therefore, not used by contractors.

C. Miscellaneous Species: Menidia menidia (Atlantic Silverside), Fundulus heteroclitus (Rollifish). Species can be studied but not routinely at this point. Could use more development. Also requires spawning and holding conditions.

IX. Laboratory Bioaccumulation Studies on Molluscs

These methods are not generally being used by contractors. The methods are well developed for a wide variety of species (i.e., oysters, scallops, mussel, quahog, etc.). Methods are currently being prepared for Ocean Disposal Manual to be published in January 1978. Methods apply to a wide variety of toxicants both in the presence and absence of substratum.

X. Benthic Bioassays using Polychaetes

In house flow-through methods for Neanthes and Arenicola are available but not currently being used outside research facility. Methods will become available in January 1978 in Ocean Disposal Manual. Chronic and acute measures in sediment system also useful in dredge spoils.

XI. Field Assessment Techniques using Mytilus edulis

Mussel Watch Program of ERL-Narragansett using biological measure of pollutant accumulation from water. Method details currently being prepared for publication. Has major use as monitoring tool in hazard assessment.

XII. Benthic Bioassay for Sediment

New technique with details in Dredge Soil Manual.

The General Methods described above highlight principal assessment tools available. There are potentially many variations within any category. Sources of method descriptions are:

1. Environmental Protection Agency-660/3-75-009. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians.

2. Environmental Protection Agency-660/9-76-010. Bioassay Procedures for the Ocean Disposal Permit Program.

3. Marine Technology Society. 1974. Marine Bioassay. Workshop Proceedings.

4. EPA/Army Corps of Engineers. 1977. Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters.

FRESHWATER ENVIRONMENT TESTING

I. Acute Toxicity Tests (static and flow-through)

Routinely used by many laboratories with many species of fish, macroinvertebrates and amphibians. (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975.)

II. Life Cycle Toxicity Tests (survival, growth, and reproduction)

Routinely used by many laboratories with Daphnia magna, fathead minnow, and brook trout. Has been successfully conducted by some laboratories with bluntnose minnow, bluegill sunfish, green sunfish, Daphnia pulex, rainbow trout, midge, mayfly, flagfish, and catfish.

III. Embryo-Larval Test (fish)

Has been routinely used with fathead minnows and brook trout. Can be used with almost any species of fish for which eggs are available. Results are good indicators of results of life cycle tests.

IV. Bioaccumulation

Has been routinely used with fathead minnows, brook trout, and bluegill sunfish. Can be used with almost any species of fish and some invertebrates.

V. Flavor Impairment (fish)

Has been routinely used with brook trout, rainbow trout, and catfish. Can be used with almost any species of fish over about 20 grams.

VI. Toxicity Tests with Algae

Has been routinely used with some species of algae by some laboratories.

BEHAVIORAL TOXICOLOGY

In the study of environmental toxins, there must be a concern with transient or permanent functional effects in addition to the documentation of manifest disease or morbidity. Functional deficits, more often than not, involve the central nervous system which plays a major integrating role in the physiology and function of an organism. Because of this integrative role, a toxic effect in many of the organ systems is also reflected in a change in the functioning of the nervous system. This functional capacity, however, cannot be evaluated by pathological, biochemical, or even physiological studies independent of a behavioral analysis.

The techniques with which to study the influences of chemicals on behavior are available largely from psychopharmacology. The somewhat standardized approach to central nervous system (CNS) drug screening in the pharmaceutical industry may not transfer directly or totally to the study of environmental agents. Weighing risks against therapeutic benefits requires different decisions for an agent intended for use under limited conditions for a relatively prescribed time, than for a chemical that might be dispersed widely, linger for many years, and expose large segments of the population.

A decision tree can be constructed to provide some guidelines for a sequential testing protocol for behavioral effects. The first step in this sequence should be an elementary screening program to determine approximate dose response functions for readily observable CNS effects. This screen can be adopted for both acute and subchronic studies. The elements in the screen must be determined by the class of compounds to be studied but might include:

(a) Activity changes: locomotion of a standard distance; spontaneous or exploratory behavior, abnormal gait.

(b) Objective signs: tremors, ptosis, salivation, convulsions, defecation, etc.

(c) Reflex changes: hyper- or hyporeflexia of cornea and pinna righting.

(d) Elicited responses: gasping, hanging, nose or tail pinch, orientation to external stimulus.

(e) Body weight changes: immediate and brief weight losses; failure to grow in relation to species and colony norms.

Instead of stopping with these observations, however, the evaluation of an environmental agent should continue for possible delayed effects. Many agents (for example, the heavy metals) do not reveal their damage immediately after exposure but only following a prolonged latent period. Any agent that

manifests delayed or residual toxic effects must be considered for rejection because of the possibility of exposing a large segment of the population. Individuals with dormant damage may be less capable of withstanding further CNS deterioration as might result from normal phenomena such as aging. Many elements in the preliminary screen can be used repeatedly to look for delayed or persistent effects.

Prenatal exposure is another potential danger with environmental agents. The combination of an immature, developing nervous system with a lack of adequate detoxification mechanisms makes the fetus and neonate especially susceptible. Sometimes the consequences of such exposure can remain dormant and not manifest themselves until later life as a behavioral disorder, mental deficiency, or overt functional impairment. Determination of long-term or delayed effects of a particular developmental influence on biological or behavioral functions requires the use of a longitudinal research design. This protocol follows specific individuals from birth through maturity. A series of standardized tests appropriate for such longitudinal studies has been published by J. Werboff in Principles of Psychopharmacology, edited by W. G. Clark and J. Del Guidice (1970, New York, Academic Press). It is strongly recommended that even chemicals that show no obvious CNS effects on the preliminary screen be studied for delayed and, especially, prenatal effects. Particularly if the effects are delayed--so a long latent period supervenes between exposure and effect--the chemical must be presumed to offer hidden dangers that, except in rare instances, will not be worth contending with, despite many other possible benefits.

If CNS effects are revealed in the preliminary screen, they should be characterized more precisely and more quantitatively, yet with minimal instrumentation and training time. This behavioral assay might include: (a) quantitative measures of spontaneous motor activity using photocell cage or an activity wheel; and (b) stable Sidman avoidance performance. The latter procedure is especially useful since the performance of the untreated animal does remain stable and reproducible over many months of testing. An avoidance procedure that uses a warning stimulus can be used as a first approximation to the study of sensory function.

An analysis of the nature of specific behavioral deficits permits some guess at the site or mechanisms of toxic action and may provide valuable suggestions as to which behavioral parameters should be monitored in exposed human populations. An investigation in depth, however, requires a substantial investment in time, talent, and money. Sometimes, of course, such an assessment is inescapable, as in the case of chemicals that get into the food chain.

Sensory function

Analysis of sensory function cannot be done independently of behavioral tests. All facets of visual functioning, viz, pattern and brightness discrimination and hue, acuity, scotopic and photopic curves, can and have been studied in a wide variety of animal species. Similar tests are available for auditory and tactile discrimination. The techniques have been important tools for studying various drugs (kanamycin and neomycin for their ototoxic

effects; pheniprazine for producing red-green color-blindness; and methyl mercury for its constriction of visual fields). Many of these techniques have been described in Animal Psychophysics, edited by W. Stebbins (1970, New York, Appleton, Century and Crofts).

Motor control

Behavioral methods for the detection of gross changes in motor control and coordination are well documented in the pharmacological literature. Two examples of methods that can be used to assess environmental toxicants are rotorod (length of time an animal can remain on a rotating rod) and time an animal can remain suspended from an inverted screen.

A more sensitive system for assessing fine motor control in animals has been developed by John Falk and his associates (Physiology and Behavior, 1969, V4, 421-427). A strain gauge is used to measure force exerted on a lever and the force output must remain within a prescribed bandwidth. Concurrent electromyograms (EMG) can also be measured.

Learning and memory

Learning and memory are critically important functions in the discharge of human affairs. Any significant disruption of these functions can have serious consequences on the quality of human life.

Maze learning has been used widely to study the effects of many variables such as cretinism, heavy metals, cerebral lesions, pesticides, anesthetic gases.

The delayed match-to-sample task (D'Amoto, The Psychology of Learning and Memory, edited by G. Bower) has been used extensively to study memory in both animals and humans, especially children. It has been used to evaluate marijuana constituents, nicotine, heavy metals, and extra low frequency magnetic fields.

Affective behaviors

Many early effects of environmental poisoning have been reported as vague, non-specific subjective complaints reflecting emotional lability. In psychopharmacology, three basic procedures have been used to assess the effects of anxiety in experimental animals: (1) Sidman avoidance conditioning; (2) the Estes-Skinner conditioned anxiety procedure; and (3) the Geller-Seifter conflict procedure. Whether these techniques are applicable to the study of environmental toxins remains to be determined.

The analysis of behavior is still an infant science and most of its procedures have not been standardized with respect to specific application. By and large, the procedures have been sufficiently delineated to be used by various laboratories with consistent, reproducible results. Mention of specific procedures should not be taken as an endorsement but rather as one portal of entry into a particular problem area.

REPORT ON
IN VITRO TESTING

Since the membership of this Working Group was limited, and the amount of time available was also limited, it was felt that the most profitable utilization of time would be to document the current references with regard to short-term assays for mutagenicity and oncogenicity and to make recommendations to the Office of Toxic Substances regarding the course of action that will enable them to meet their goals in these areas. Short-term tests for cytotoxicity were also briefly discussed.

USE OF SHORT-TERM TESTS FOR EVALUATION OF MUTAGENIC POTENTIAL

It was the general consensus of the committee that there are several adequate references available documenting the current status of the use of short-term tests to assess potential mutagenic hazards of chemicals (1-7). In particular, four documents (4-7), either completed or nearing completion, represent the efforts of several committees, and it was generally felt that this area has been reasonably explored and appropriately documented.

The available procedures for detecting and characterizing chemical mutagens are probably as good if not better than other toxicological endpoints in that not only can a mutational process be directly detected, but frequently an understanding regarding the mechanism(s) of action of the mutagen can be determined on a molecular level. It was noted that since toxicological decisions in general are usually based upon non-human studies, it is reasonable to apply the same criteria to decisions in the area of mutagenicity. It is thus recommended that chemical mutagenesis testing should be an integral part of any toxicological evaluation of chemicals and should form a portion of the data upon which regulatory decisions be made.

USE OF SHORT-TERM TESTS FOR EVALUATION OF CARCINOGENIC POTENTIAL

Studies utilizing short-term assays for oncogenicity have been extensive, and in general a good correlation between positive results in various systems to known carcinogenicity in vivo has been found. However, an in-depth evaluation of the various methodologies has not yet been undertaken by an expert committee. Several expert committees have assessed the usefulness of short-term tests in evaluating the potential mutagenicity of chemicals (as discussed above). Although there is considerable overlap between evaluating the status of short-term tests for predicting mutagenicity and oncogenicity, several areas involved in assessing the value of short-term tests for predicting carcinogenicity of chemicals have not yet been approached by any expert committee: (a) It can be determined if a

chemical is really a carcinogen by testing it in animals (there are also data in humans for about 20 carcinogens). Thus the ability of the various short-term tests to detect carcinogens should be checked. (b) There are several types of short-term tests for carcinogenicity, notably in vitro transformation tests, which have not been evaluated by the expert committees on mutagenesis since their endpoints are not mutations. (c) The problem of how short-term tests for carcinogenicity should fit into the overall scheme of the toxicological evaluation of regulated substances is again a problem that has not been considered. Since animal carcinogenicity tests remain the best indicators of the potential human carcinogenicity of chemicals, it is essential to determine how short-term test results influence whether or not an animal cancer test is done, and how short-term test results are weighed against animal cancer test data in the overall evaluation of the potential human hazard of a chemical.

These are decisions which should ultimately be made at policy-making levels within the Agency. However, an analysis of the scientific background relevant to these decisions is crucial to assist the policy makers in coming to reasonable and scientifically credible decisions. It was strongly urged that such an in-depth study be undertaken. It was also noted that there have been recent reviews on in vitro studies for oncogenesis which may be particularly pertinent (8-9) and that certain organizations such as the Food and Drug Administration and the Clearing House on Carcinogens at the National Cancer Institute are planning to undertake evaluation of the current status o the methodologies and results in these areas. However, until such an overall evaluation is completed by these or similar organizations, the relative importance of various assays for predicting carcinogenicity in vivo, and particularly in man will remain undetermined. It was emphasized, nevertheless, that there are sufficient data to make such an evaluation at the presen time.

It was the consensus of the group that although a number of tests are available as an aid in indicating potential carcinogenic hazards, such tests cannot at present stand alone as identifying a substance as a carcinogen, i.e., long-term animal carcinogenicity studies are still necessary. It was further recognized that areas of cocarcinogenesis and promotion of oncogenesis need further research. There is a very small data base in these areas.

CYTOTOXICITY

Although cytotoxicity studies were not explored at length it was noted that systems for evaluation of acute cellular toxicity in cell culture have provided useful information regarding the potential in vivo activity of certain classes of agents such as antitumor drugs, prosthetic materials and devices, biomedical implants, and antibiotics. Such tests consider transient (reversible) morphological and biochemical alterations as well as irreversibl effects leading ultimately to cellular destruction. These changes can be quantitated and hence permit reasonable estimates of cytotoxic potency. Although it is obviously impossible to replicate the entire range of cellula responses of the intact animal by the use of tissue culture methods, certain target cell types utilizing activation systems can be maintained in vitro

and provide useful information regarding cytotoxicity and metabolism.

RECOMMENDATIONS OF THE WORKING GROUP TO THE OFFICE OF TOXIC SUBSTANCES

1. Convene a group of experts to consider specifically the applicability of the Pesticide Guidelines to the need of the Office of Toxic Substances in using short-term tests as indicators of mutagenic potential of chemicals.

2. Interact with the other regulatory agencies (FDA, OSHA, CPSC) in the development of a technology assessment document (along the lines of the EPA Office of Pesticide Guidelines, and the CPSC and DHEW mutagenicity documents), to evaluate the status and use of short-term tests as aids in evaluating the carcinogenic potential of chemicals.

It should be emphasized that this committee felt strongly that its only functions could be those of reviewing and acknowledging those documents which should provide significant source material for the EPA and others, as well as making certain suggestions regarding further needs, such as the formation of more complete documents on short-term oncogenicity studies.

REFERENCES

1. World Health Organization. 1974. Assessment of the Carcinogenicity and Mutagenicity of Chemicals. Technical Report Series No. 546.

2. Health and Welfare Ministry Canada. 1975. The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity.

3. J. W. Drake, Chairman, Prepared by Committee 17 of the Environmental Mutagen Society. 1975. Environmental Mutagenic Hazards. Science, 187:503-514.

4. Working Group of the Sub-Committee on Environmental Mutagenesis. 1977. Approaches to Determining the Mutagenic Properties of Chemicals: Risk to Future Generations. Prepared for the DHEW Committee to Coordinate Toxicological and Related Programs.

5. Office of Pesticides Program, Environmental Protection Agency, Draft, July 12, 1977. Mutagenicity Testing Requirements Section of the FIFRA. Registration Guidelines for Hazard Evaluation of Humans and Domestic Animals.

6. Office of Pesticides Program, Environmental Protection Agency, Draft, August 31, 1977. Criteria for Evaluating the Mutagenicity of Chemicals.

7. National Academy Sciences. 1977. Principles and Procedures for Evaluating the Toxicity of Household Substances. Chapter VI, Mutagenicity Tests.

8. Montesano, R., H. Bartsch, and L. Tomatis, eds. 1976. Screening Tests in Chemical Carcinogenesis. International Agency for Research on Cancer, Lyon, IARC Scientific Publ. No. 12, 1976.

9. U. Saffiotti and H. Autrup, eds. 1977. In Vitro Carcinogenesis, Guide to the Literature, Recent Advances and Laboratory Procedures. National Cancer Institute Carcinogenesis Report Series, DHEW Publication No. (NIH) 78-844.

REPORT ON
MODEL ECOSYSTEMS

MICROCOSMS AS SCREENING TOOLS FOR TOXIC CHEMICALS

DEFINITION

Microcosms are relatively small experimental units that contain the major components and exhibit the major processes of natural ecosystems. Conceptually, microcosms are functionally similar to the natural ecosystems they represent; however, the two may differ in origin and structure. Microcosms may be constructed artifically from stock components or, intact, co-adapted communities and their abiotic substrates may be excised from a natural system (1). From the outset, it should be realized that microcosms should not be expected to provide initial assessments of chemical hazards in the environment. Initial assessments and hazard rankings of chemicals might best be accomplished using available chemical benchmark information, simple short-term laboratory tests (e.g., lethality, EC_{50}, LD_{50}), and predictive mathematical models of chemical behavior. Information gathered from these initial assessments of potential chemical behavior in the environment can aid in the design of subsequent microcosm studies, and in the interpretation of microcosm-derived data. It is not expected that microcosm studies will be used to provide information on the toxic effects of all chemicals. However, they may provide information on the chronic, long-term effects of chemicals on fundamental ecosystem processes (e.g., energy flux, nutrient cycling, homeostatic properties, species interactions).

ADVANTAGES OF MICROCOSMS

Microcosm experimental units afford the researcher control of ecosystem complexity relating to both biotic and abiotic ecosystem components. They are low in cost and easily replicable from a given ecosystem, and allow control of selected environmental conditions, such as temperature, humidity or light. Microcosms are attractive since they obviate the need for contamination of natural environments (2). Microcosms fall into two general categories: (1) Process-oriented (simple) microcosms, and (2) Integrative (complex) microcosms. Simple microcosms allow the measurement of chemical transfer rates and coefficients over short experimental time periods. However, they may provide only order of magnitude estimates of more complex systems. Complex microcosms, containing more of the functional components of natural ecosystems, include more of the complexity and events that may be caused by biotic-biotic and biotic-abiotic interactive processes.

DISADVANTAGES OF MICROCOSMS

There is a real difficulty in extrapolating results from processes measured in isolation to actual environmental behavior of chemicals. It is also difficult to evaluate the comparability of similar, but not-identical microcosm systems (need for cross comparison). Microcosms are generally tailored to specific ecosystems and specific questions about ecosystem processes and chemical behavior. Therefore, results obtained from them are usually not amenable to generalization of other ecosystems. It is expected that microcosms will best be used to evaluate specific situations rather than as a general screening tool.

Environmentally, realistic microcosms should be expected to exhibit parameter variability the same as that exhibited by the natural environment. Currently, it is unknown to what degree ecosystem function is distorted by fabrication or field excision of microcosms, and accepted methods for extrapolation to field situations do not exist.

PROCESSES AMENABLE TO STUDY BY MICROCOSM INVESTIGATION (3)

1. Mobility
    a. Physical transport pathways
    b. Biological uptake, accumulation, food chain biomagnification
2. Partitioning Among Microcosm Components
3. Degradation to Simple Substances
    a. Abiotic and physicochemical
    b. Biotic and metabolic
4. Transformation
    a. Physicochemical (photolysis, redux reactions)
    b. Biogenic Transformation
5. Ecosystem Effects
    a. Behavior of organisms (populations)
    b. System metabolism (energy, primary production (P), respiration (R), P/R ratio, carbon dioxide evolved oxygen utilized)
    c. Nutrient cycling
    d. Community dynamics (multi-species interactions)

UTILITY OF MICROCOSMS IN TOXIC COMPOUND SCREENING APPROACH

It is strongly emphasized that the lack of any "standard microcosm" underscores the requirement that the microcosm itself address specific questions and data needs.

Microcosm experiments are intended to bring out results to be expected only when separate components are combined into a system. Lowered magnitudes of responses may result from the buffering effect of the presence of many components, or increased responses may result from multiple pathways or cumulative effects. Were it not for the possibility of unforeseeable results, there would be little reason for testing toxic chemicals in microcosms.

Interpretation of results from microcosms involves at least changes in scale. Components of microcosms are present because they are considered to be important components in real world systems. Yet it is virtually impossible to include these components in the same proportion to each other as expected in any real world system. Therefore data on behavior of chemicals and responses of organisms must be differentially scaled when the results are applied to the real world. We term this scale of results "projection."

Although we emphasize that no "standard" is now accepted or proposed, there are traditional studies which parallel the microcosm concept. For example, pond studies of productivity, weed control or, in some cases, chemical compound effects have been conducted for decades and give some background for methodology and statistical analysis.

An appropriate microcosm experimental approach should then be designed, phased, and sampled according to previously identified extrapolation mechanisms. Microcosm testing or screening lends itself to certain kinds of questions of effects or fate of specific compounds. It is important to design the microcosm test in terms of real world environmental types, so that results of this operation bracket real world questions in a relevant or relatable way. These questions of relevance guide in the kind or dosing of the microcosm test applied.

Examples of the kinds of questions to be considered include:

1. Characteristics of toxic substances to be tested.
2. Processes to be considered in the test (e.g., transformation, transport, uptake or partitioning, population impacts, etc.).
3. The ecosystem(s) of concern which microcosm(s) test(s) should address; in other words, the breadth of environmental concern involved.
4. What "scale" of microcosm test is most appropriate?
5. What duration(s) should test be run?

The next question should be considered before executing the test:

6. What interpretation mechanisms should be used to extrapolate results from test to prediction or estimation of real world environments?

Projection of microcosm outputs may be accomplished in at least two ways:

Subjective projection: interpretation of microcosm outputs in a direct manner assuming that the microcosm used is an accurate representation or simulation of its real world counterpart. This requires careful calibration of microcosm parameters to environmental counterparts. These data still become weighed, prioritized and integrated into the decision matrix of the regulatory user, and are therefore subjectively judged with toxicological, socioeconomic or other factors in producing the decision or recommendation.

Use of models in place of a subjective interpretation mechanism does not fundamentally change the role of the regulator. Information enters the decision matrix also to be evaluated along with toxicity, socioeconomic and other factors. Model-derived information is merely more specific and somewhat more quantitative; therefore, it can be weighed more objectively.

Objective projection: an alternative technique, objective projection, is to use mathematical models representing the fate of the material in the microcosm as a tool for projection. Such a model would be based upon physical, chemical, and biological characteristics of the chemical, together with description of the microcosm. Models of this sort can reasonably well predict the fate of the chemical in the microcosm. Change of scale of the environmental factors to describe an environment of interest will then yield a projection of the microcosm results to that environment. In this manner results of microcosm studies can be much more widely applied.

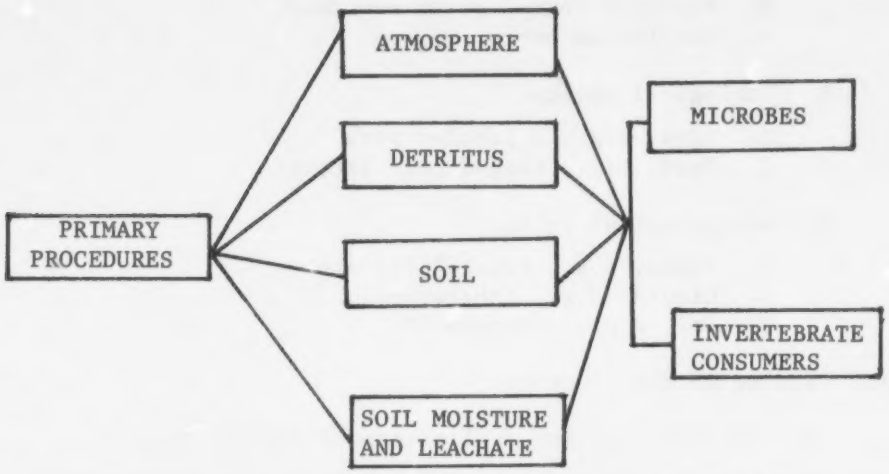
It should be emphasized that while prediction of fate by models based upon physical chemical principles is feasible, there are few principles that can be employed to predict effects. To the extent that dose-response relationships exist, however, expected effects can be computed based upon results predicted by models.

Chemical fate simulation should focus on conceptualization of the systems being studied and on the development of a set of equations for the description and prediction of major chemical transport pathways among ecosystem and microcosm components. A preliminary model for chemical transport in a terrestrial ecosystem is shown on the following page.

This model is, of course, subject to revision as experimental results become available to validate the essentiality of each component. It is expected that not only will the experimental results influence the conceptual model but the set of equations depicting transport and transformation will influence experimental design.

The sensitivity of model parameters and hypothesized chemical transport between system components may be evaluated with critical pathway analyses. In addition, sets of equations describing transport may be solved in order to compare predicted compartmental values with time-series measurements of microcosm parameters. With replication of microcosms, the chemical transport models developed may be validated independently of the data used to estimate the model parameters. Transport models may also be used in comparative-predictive evaluation of large-scale natural ecosystem models similar

to those developed under other programs. (2,4,5)



(Modified from Draggan 1976) (2)

Many types of microcosms and methods of study have been developed for assessing chemical behavior in various ecosystems. Tables 1 and 2 give examples of applications. (3)

TABLE 1. TYPES OF MICROCOSMS

Terrestrial
Terrestrial - Aquatic
River
Aquatic - Batch
Aquatic - Continuous Culture
Chemostate and Turbiodostat
Special Microcosms
Species Defined (Gnotobiotic)
In situ Bioassay
Closed (Biorengenerative Life Support)
Naturally Occurring Microcosms

TABLE 2. METHODS COMMONLY USED IN MICROCOSM STUDIES

I. Types of Inputs

A. Solution

1. Single entry, point source
2. Single entry, mixed into system

(Continued)

TABLE 2. METHODS COMMONLY USED IN MICROCOSM STUDIES (Continued)

3. Multiple entry, point or mixed
4. Continuous entry

B. Biological entity

1. Live, e.g., a labeled prey
2. Dead, e.g., tagged leaf litter

C. Nonbiological entity

1. Solid, e.g., labeled fly ash
2. Liquid, e.g., labeled rain
3. Gas, e.g., nitrogen-15

II. Timing of Measurements

A. Initial sampling (validation of initial input)

B. Periodic sampling, using subsamples of replicate(s)

C. Terminal sampling on portion of the replicates (e.g., destructive sampling of 1/3 of the replicates at 3 different durations)

D. Terminal sampling of all replicates simultaneously

III. Compartments Measured

A. One [i.e., species of interest (fish) or system output (leachate)]

B. Special

C. All compartments except container surfaces and atmospheric gases

D. All compartments including container surfaces and atmospheric gases

IV. Entity Measured per Compartment

A. Chemical of interest (e.g., radionuclide or DDT)

B. Carrier (or stable isotope) mass

C. Degradation products (e.g., DDD, DDE)

D. Compartment mass (e.g., dry weight)

V. Calculated Values

A. Concentration of entity in a compartment

B. Transfer rates between compartments (first order,

(Continued)

TABLE 2. METHODS COMMONLY USED IN MICROCOSM STUDIES (Continued)

	function of mass in donor compartment; second order, function of mass in donor and recipient compartments) requiring sequence of measurements and model of interactions
C.	Specific activity
D.	Concentration factor, also called biological or ecological magnification, bioaccumulation factor or index (ratio of concentration in recipient compartment/donor compartment--donor may be water or food)
E.	Biodegradability index (ratio of concentration of breakdown productions/parent compounds, e.g., polar/nonpolar labeled compounds, Metcalf et al., 1971b)
F.	Distribution among major compartments, e.g., % in soil, water, algae, snails
G.	Total budget studies
VI.	Special Types
A.	Species defined (gnotobiotic)
B.	In situ bioassay
C.	Naturally occurring microcosms
D.	Closed (bioregenerative life support)

REFERENCES

1. Draggan, S., and J. M. Giddings. 1978. Testing toxic substances for protection of the environment. Sci. Total Environ., 9:63-74.

2. Draggan, S. 1976. The microcosm as a tool for estimation of environmental transport of toxic materials. Inter. J. Environ. Studies, 10:65-70.

3. Witherspoon, J. P., E. A. Bondietti, S. Draggan, F. B. Taub, N. Pearson, and J. R. Trabalka. 1976. State-of-the-Art and Proposed Testing for Environmental Transport of Toxic Substances, Report No. EPA-560/5-76-001. U.S. EPA, Office of Toxic Substances, Washington, D.C., 164 pp.

4. Sollins, P. 1971. A Computer Program for Modelling Ecological Systems. ORNL-IBP-71-5. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

5. Goldstein, R. A., and J. W. Elwood. 1971. A two-compartment, three parameter model for the absorption and retention of ingested elements by animals. Ecology, 52:935-939.

GENERAL BIBLIOGRAPHY

Alexandrov, V. A. 1972. Embryotoxic and Teratogenic Effects of Chemical Carcinogens. International Agency for Research on Cancer (IARC). IARC Scientific Publications, No. 4. Transplacental Carcinogens. pp. 112-126.

Ames, B. N. 1974. A combined bacterial and liver test system for detection and classification of carcinogens as mutagens. Genetics, 78:91-95.

Ames, B. N., J. McCann and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364.

Anonymous. 1973. The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity. Health and Welfare Ministry, Canada.

Anonymous. 1977. Is a single cancer test policy feasible? Chemical Week, (July 13, 1977):49-50.

Balazs, T. 1976. Assessment of the Value of Systemic Toxicity Studies in experimental animals. In: Advances in Modern Toxicology, Vol. 1, Pt. 1. John Wiley and Sons, New York. pp. 141-153.

Bartsch, H. 1976. Mutagenicity Test in Chemical Carcinogenesis. IARC Scientific Publications No. 13. Environmental Pollution and Carcinogenic Risks. Lyon. pp. 229-240.

Bimboes, D., and H. Greim. 1976. Human lymphocytes as target cells in a metabolizing test system in vitro for detecting potential mutagens. Mutation Res., 35:155-160.

Brusick, D., R. J. Wier and H. E. Gis. 1976. Screening Program for the Identification of Potential Chemical Mutagens and Carcinogens. Litton Bionetics, Kensington, Maryland 20795 (301) 881-5600.

Cole, L. K., R. L. Metcalf and J. R. Sanborn. 1976. Environmental fate of insecticides in terrestrial model ecosystems. Intern. J. Environ. Studies, 10:7-14.

Collins, T. F. X., and E. Collins. 1976. Current Methodology in Teratology Research. In: Advances in Modern Toxicology, Vol. 1, Pt. 1. John Wiley and Sons, New York. pp. 155-171.

Cox, R., W. K. Masson, and D. A. Bance. 1976. An alternative to petri dishes in quantitative mutation experiments with cultural mammalian cells. Mutation Res., 35:173-178.

Crow, J. F., and S. Abrahamson. 1971. Drosophila Methods for Mutagenicity Testing. In: Drugs of Abuse, S. S. Epstein, ed. M.I.T. Press, Cambridge, MA. pp. 136-139.

de Serres, F. J. 1971. Microbial Methods for Mutagenicity Testing. In: Drugs of Abuse, S.S. Epstein, ed. M.I.T. Press, Cambridge, MA. pp. 127-135.

de Serres, F. J. 1976. Strength and Weaknesses of Microbial Test Results for Predicting Human Response. Preprint Conf. on the Status of Predictive Tools and Application Safety Evaluation, Little Rock, Ark., Nov. 17-19, 1976.

Draggan, S. 1978. The microcosm: Biological Model of the Ecosystem. Monitoring and Assessment Research Centre (MARC). Monitoring and Assessment Research Centre, London, U. K. (In Press).

Drake, J. W., Ch. 1975. Committee 17, Environmental Mutagen Society. Environmental Mutagenic Hazards. Science, 187:503-514.

Environmental Carcinogenesis Subcommittee, National Cancer Advisory Board. 1975. Draft of Criteria for Establishing the Carcinogenicity of Chemicals.

Environmental Health Sciences Center of O.S.U. 1977. Terrestrial Microcosm Workshop Handbook, Oregon State University, Corvallis, 51 pp.

Environmental Mutagenesis Subcommittee. DHEW Committee to Coordinate Toxicology and Related Programs. 1977. Approaches to Determining the Mutagenic Properties of Chemicals: Risk to Future Generations.

EPA. 1977. IERL-RTP Procedures Manual: Level 1. Environmental Assessment Biological Tests for Pilot Studies. EPA 600/7-77-043.

European Environmental Mutagen Society. 1977. General Principles and Minimal Criteria for Mutagenicity Screening. 8 pp.

Fahrig, R. 1975. Development of host-mediated mutagenicity tests-yeast systems II. Recovery of yeast cells out of testes, liver, lung and peritoneum of rats. Mutation Res., 31:381-394.

Ferens, M. C., and R. J. Beyers. 1972. Studies of a simple laboratory microecosystem-effects of stress. Ecology, 53(4):709-713.

Fishbein, L. 1977. Potential Industrial Carcinogens and Mutagens. U.S. Environmental Protection Agency Report, EPA-560/5-77-005.

Flamm, W. G. 1974. A tier system approach to mutagen testing. Mutation Res., 26:329-333.

Flamm, W. G. 1975. Test systems for assessing mutagenic potential of chemical substances. J. Assoc. Off. Anal. Chem., 58:668-671.

Giddings, J. M., and G. K. Eddlemon. 1978. The effects of microcosm size and substrate type on aquatic microcosm behavior and arsenic transport. Arch. Environ. Contain. Toxicol., 6:491-505.

Gillett, J. W., J. R. Hill, A. W. Jarvenin and W. P. Schoor. 1974. A conceptual model for the movement of pesticides through the environment. U.S. Environmental Protection Agency Ecological Research Series, EPA 600/3-74-024, 79 pp.

Gillett, J. W., and J. D. Gile. 1976. Pesticide fate in terrestrial laboratory ecosystems. Intern J. Environ. Studies, 10:15-22.

Goldberg, L., ed. 1974. Carcinogenesis Testing of Chemicals. CRC Press, 18901 Cranwood Parkway, Cleveland, Ohio 44128.

Green, S., E. Zieger, K. A. Palmer, J. A. Springer, M. S. Legator. 1976. Protocols for the dominant lethal test, host mediated assay, and in vivo cytogenetic test used in the FDA review of substances in the GRAS List. J. Tox. and Env. Health, 1:921-928.

Green, S., F. M. Moreland and W. G. Flamm. 1977. A new approach to dominant lethal testing. Tox. and Appl. Pharm., 39:549-552.

Harris, R. C. 1976. Suggestions for the development of a hazard evaluation procedure for potentially toxic chemicals. Report No. 3, Monitoring and Assessment Research Centre, Chelsea College, London. 18 pp.

Hayes, W. J., Jr. 1972. Tests for Detecting and Measuring Long-Term Toxicity. In: Essay in Toxicology, Vol. 3., W. J. Hayes, Jr., ed. pp. 65-67.

Hoel, D. G., D. W. Gaylor, R. L. Kirschstein, U. Saffiotti, and M. A. Schneiderman. 1975. Estimation of risks of irreversible delayed toxicity. J. Tox. and Env. Health, 1:133-151.

Hsu, T. 1976. Editorial. Mammalian Chromosome Newsletter, 17:1-6.

Interagency Testing Committee. 1977. Preliminary List of Chemical Substances for Further Evaluation, by the Toxic Substances Control Act Interagency Testing Committee.

International Agency for Research of Cancer (IARC). 1974. Chemical Carcinogenesis Essays. R. Montesano and L. Tomatic, eds. IARC Scientific Publications No. 10. Lyon.

International Agency for Research of Cancer (IARC). 1976. Screening Tests in Chemical Carcinogenesis, R. Montesano, H. Bartsch and L. Tomatis, eds. IARC Scientific Publications No. 12. Lyon.

Isensee, A. R. 1976. Variability of aquatic model ecosystem derived data. Intern. J. Environ. Studies, 10(1):35-41.

Krueger, J. 1970. Statistical Methods in Mutation Research, In: Chemical Mutagenesis in Mammals and Man. F. Vogel and G. Rockborn, eds. Springer-Verlag, N. Y. - Heidelberg.

Leehouts, J. W. 1977. TSCA - a Symposium. Chemteck, 1977:408-411.

Leonard, A. 1975. Tests for heritable translocations in male mammals. Mutation Res., 31:291-298.

Levin, S. A. 1976. Population dynamic models in heterogenous environments. Ann. Rev. Ecol. Syst., 7:287-310.

Librizzi, W. 1977. TSCA - an EPA View. Chemteck, 1977:404-408.

Maugh, Thomas H., II. 1977. Biochemical markers. Early warning signs of cancer. Science, (5 Aug. 1977):543-545.

Mayer, V. W., and W. G. Flamm. 1975. Legislative and technical aspects of mutagenicity testing. Mutation Res., 29:295-300.

Metcalf, R. L. 1978. Partition coefficient and environmental fate. Proc. Symp. Terrestrial Microcosms Environ. Chem., 13-17 June 1977. Oregon State University, Corvallis, (In Press).

McNamara, B. P. 1976. Concepts in Health Evaluation of Commercial and Industrial Chemicals. In: New Concepts in Safety Evaluation. M. A. Mehlman, R. E. Shapiro and H. Blumenthal, eds. John Wiley & Sons, New York, pp. 61-104.

National Academy of Sciences. 1975. Principles for Evaluating Chemicals in the Environment. NAS, Washington, D. C.

National Science Foundation. 1975. Final Report of NSF Workshop Panel to Select Organic Compounds Hazardous to the Environment. NSF, Washington, D. C.

NCI. 1976. Carcinogenesis Bioassay Program-Specifications and Standards. Tracor Jitco, Inc., Rockville, Maryland.

Odum, E. P. 1977. The emergence of ecology as a new integrative discipline. Science, 195:1289-1293.

Office of Pesticide Programs, Environmental Protection Agency. 1977. Draft Testing Program. 162.60.23-30 Chemistry Requirement: 162.70.1-13 Toxic Hazard Evaluation Wildlife and Aquatic Organisms: 162.80.3-5, 162.81.1-8, 162.82.1-4, 162.83.1 Humans and Domestic Animals: 162.83.2 Oncogenicity Studies: 162.83.3-4, 162.85.1 Teratogenicity Studies.

Office of Pesticide Programs, Environmental Protection Agency. 1977. Draft Mutagenicity Testing Requirements Section, FIFRA, Section 3, Guidelines on Hazard Evaluation of Humans and Domestic Animals. (July 12, 1977).

Office of Pesticide Programs, Environmental Protection Agency. 1977. Criteria for Evaluating the Mutagenicity of Chemicals. (Draft)

Office of Toxic Substances, U. S. Environmental Protection Agency. 1976. Draft Survey and Evaluation of In Vitro Toxicity Test Methods. G. Woodard, ed.

Office of Toxic Substances, U. S. Environmental Protection Agency. 1977. Draft Assessment and Control of Chemical Problems. An Approach to Implementing the Toxic Substances Control Act.

Public Law 94-469-October 11, 1976. Toxic Substances Control Act.

Roehrborn, G. 1972. Correlation Between Mutagenesis and Carcinogenesis. International Agency for Research on Cancer (IARC). IARC Scientific Publications, No. 4. Transplacental Carcinogens. pp. 168-174.

Sassoon, H. F. 1976. Survey and Evaluation of Techniques Used in Testing Chemical Substances for Teratogenic Effects. Tracor Jitco, Inc., Rockville, Maryland.

Schmid, W. 1975. The micronucleus test. Mutation Res., 31:9-15.

Searle, A. G. 1975. The specific locus test in the mouse. Mutation Res. 31:277-290.

Shubik, P., and O. B. Clayson. 1976. Application of the Results of Carcinogen Bioassays to Man. IARC Scientific Publications No. 13. Environmental Pollution and Carcinogen Risks. Lyon. pp. 241-252.

Sivak, Andrew. 1976. Letters: The Ames assay. Science, (July). 193.

Sontag, J. M., N. P. Page and U. Saffiotti. 1976. Guidelines for Carcinogen Bioassay in Small Rodents. National Cancer Institute Carcinogenesis Technical Report Series, No. 1. NCI-CG-TR-1: DHEW No. (NIH) 76-801.

Sperling, F., and J. L. McLaughlin. 1976. Biological parameters and the acute LD_{50} test. J. Assoc. Off. Anal. Chem., 59:734-736.

Stitch, H. F., P. Lam, L. W. Lo, D. J. Koropatnick, and R. H. C. San. 1975. The search for relevant short term bioassays for chemical carcinogens: the tribulation of a modern Sisyphus. Can. J. Genetic and Cytology, 17:471-492.

Stockinger, H. E. 1977. The case for carcinogen TLV's continues strong. Occupational Health and Safety, (March/April 1977):54-58.

Stolz, D. R., L. A. Poirier, C. C. Irving, H. F. Stitch, J. H. Weisburger and H. C. Grice. 1974. Review article: evaluation of short-term tests for carcinogenicity. J. Tox. and Appl. Pharm., 29:157-180.

Tomatis, L. 1976. Extrapolation of Test Results to Man. IARC Scientific Publications No. 13. Environmental Pollution and Carcinogenic Risks. Lyon. pp. 261-262.

Tracor Jitco, Inc. 1977a. Sample Biological Assay Protocols
- o Chronic 24-Month Mouse Feeding Study
- o Chronic Feeding Study in Rats (In Utero Exposure)
- o Primary Dermal Irritation Study in Rabbits
- o Acute Inhalation Study in Rats
- o Subchronic Inhalation Study in Rats
- o Chronic Inhalation Study in Rats (In Utero Exposure)
- o 24-Month Inhalation Study in Mice
- o 14-Day Multiple Dose Inhalation Study in Rats
- o 1/10 Lifetime Inhalation Study in Rats
- o Mammalian Reward and Motivation Study
- o Teratogenicity in Rats
- o Teratogenicity in Rabbits
- o Three-generation Reproduction Study in Rats
- o Microbial Assay (Ames Test)
- o In-vitro Transformation of BALB/3T3 Cells
- o Mouse Lymphoma Forward Mutation Assay
- o Unscheduled DNA Synthesis (UDS) in Human WI-38 Cells

Tracor Jitco, Inc. 1977b. Short-Term Mutagenicity/Carcinogenicity. (Includes Figure Showing Hypothetical Scheme for Relating Mutagenicity and Tumorigenicity).

Weber, E., K. Bidwell and M. S. Legator. 1975. An evaluation of the micronuclei test using triethylenemelamine, trimethylphosphate, hycanthone and niridazole. Mutation Res., 28:101-106.

Weber, J. B. 1977. The pesticide scorecard. Environmental Science and Technology, 11(8):756-76.

Weil, C. S. 1968. Relationship of Results of Long-Term Toxicity Studies to Those of Shorter Duration, AMRL-TR-175. Paper No. 8:93-101.

Weil, Carrol S. 1972. Guidelines for experiments to predict the degree of safety of a material for man. J. Tox. and Appl Phar., 21:194-199.

Weil, Carrol S., N. I. Condra, and C. P. Carpenter. 1971. Correlation of 4-hour vs. 24-hour contact skin penetration toxicity in the rat and rabbit and use of the former for predictions of relative hazard of pesticide formulations. J. Tox. and Appl Pharm., 18:734-742.

Wilson, J. G. 1975. Reproduction and teratogenesis: current methods and suggested improvements. J. Assoc. Off. Anal. Chem., 58:657-667.

World Health Organization. 1974. Assessment of the Carcinogenicity and Mutagenicity of Chemicals. WHO Tech. Report Series No. 546.

Zimmering, S. 1975. Utility of Drosophila for detection of potential environmental chemical mutagens. Anal. New York Academy of Sciences, 269:26-33.

LIST OF WORKSHOP PARTICIPANTS

Name	Organization and Location
Delbert S. Barth	Office of Health and Ecological Effects, U. S. Environmental Protection Agency, Washington, D. C.
Werner F. Beckert	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
Joseph V. Behar	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
William F. Benedict	Children's Hospital of Los Angeles, Los Angeles, California
Charles A. Bicking	Tracor Jitco, Inc., Rockville, Maryland
Erich W. Bretthauer	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
John M. Bryant	Occupational Safety and Health Administration, U. S. Department of Labor, Austin, Texas
Lawrence A. Burns	Environmental Research Laboratory, U. S. Environmental Protection Agency, Athens, Georgia
Lorne A. Campbell	Tracor Jitco, Inc., Rockville, Maryland
William P. Davis	Environmental Research Laboratory, U. S. Environmental Protection Agency, Bears Bluff, South Carolina
Sidney Draggan	Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee; now National Science Foundation, Washington, D. C.
Dom V. Finocchio	Department of Pathology, University of Washington School of Medicine, Seattle, Washington
Donald E. Gardner	Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina

(continued)

Name	Organization and Location
John Gentile	Bioassay Systems Laboratory, U. S. Environmental Protection Agency, Narragansett, Rhode Island
Sidney Green	Department of Pharmacology, Howard University College of Medicine, Washington, D. C.
Harry W. Hays	Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.
Robert M. Hehir	Consumer Product Safety Commission, Bethesda, Maryland
Renate Kimbrough	Center for Disease Control, Department of Health, Education, and Welfare, Atlanta, Georgia
Robert R. Kinnison	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
John H. Kreisher	Council of Tobacco Research, New York, New York
Roy R. Lassiter	Environmental Research Laboratory, U. S. Environmental Protection Agency, Athens, Georgia
Marvin S. Legator	University of Texas Medical Branch, Galveston, Texas
John F. Lontz	Veterans Administration Center, Wilmington, Delaware
Russel Malcolm	Environmental Research Laboratory, U. S. Environmental Protection Agency, Narragansett, Rhode Island
Irving Mauer	Tracor Jitco, Inc., Rockville, Maryland
Joyce McCann	University of California, Berkeley, California
Craig McFarlane	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
Bernard P. McNamara	Chemical Systems Laboratory, Edgewood, Maryland
George B. Morgan	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
Stephen S. Olin	Tracor Jitco, Inc., Rockville, Maryland

(continued)

Name	Organization and Location
Ruth Pertel	Office of Pesticides Programs, U. S. Environmental Protection Agency, Washington, D. C.
Robert D. Rogers	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
John A. Santolucito	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
Richard E. Stanley	Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Las Vegas, Nevada
Thomas W. Stanley	Office of Monitoring and Technical Support, U. S. Environmental Protection Agency, Washington, D. C.
Charles S. Stephan	Environmental Research Laboratory, U. S. Environmental Protection Agency, Duluth, Minnesota
Robert G. Tardiff	Health Effects Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio
Richard L. Titus	Chemistry Department, University of Nevada, Las Vegas, Nevada
A. C. Trakowski	Office of Monitoring and Technical Support, U. S. Environmental Protection Agency, Washington, D. C.
William M. Upholt	Office of Assistant Administrator, Toxic Substances, U. S. Environmental Protection Agency, Washington, D. C.
Lawrence R. Valcovic	National Institute of Environmental Health Services, Research Triangle Park, North Carolina
Michael Waters	Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina
Jack A. Winstead	National Academy of Science, Washington, D. C.